

**DATA EVALUATION RECORD  
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE  
GUIDELINE OPPTS 850.1075**

1. **CHEMICAL:** Cetylpyridinium chloride (CPC) **PC Code No.:** 069160

2. **TEST MATERIAL:** Cetylpyridinium chloride (CPC) **Purity:** 99.9%

3. **CITATION**

Author: B Knight and K Paterson

Title: CPC Determination of Acute Toxicity (LC<sub>50</sub>) to Rainbow Trout (96 h, Static)

Report Date: February 10, 2005

Laboratory: Inveresk, Tranent, EH33 2 NE, Scotland

Sponsor: Rutherford Chemicals LLC

Study Report ID: #23188

Laboratory Report ID: Inveresk Project No. 804193

MRID No.: 468162-05

4. **REVIEWED BY:**

**Signature:** Richard C. Petrie, Agronomist – Team 3 Leader  
RASSB/AD/OPP/OPPTS

*R.C. Petrie*

**Date:** 1/23/08

5. **APPROVED BY:**

**Signature:** Norm Cook, Chief  
RASSB/AD/OPP/OPPTS

*Norm Cook*

**Date:** 1/23/08

6. **STUDY PARAMETERS**

**Scientific Name of Test Organism:** *Oncorhynchus mykiss*

**Age of Test Organism:** No data were provided

**Definitive Test Duration:** 96 hours

**Study Method:** Static

**Type of Concentrations:** Nominal and mean measured

**7. CONCLUSIONS****Verified Results Synopsis:****Statistical Method:** Binomial Test**Results Verification Synopsis:**96-hr LC<sub>50</sub>: 0.15 mg/L

95% C.I: 0.11-0.21 mg/L

NOEC: 0.11 mg/L by observation.

**8. ADEQUACY OF THE STUDY****A. Classification:** CORE**B. Rationale:****C. Repairability:****9. GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- § No data were provided on the age of the test organisms at receipt, but the animals were 4-6 cm long and weighed less than 3 g, which are juvenile size and weights.
- § Duplicate aliquots (*ca* 20 mL) were removed from each test tank at 0 h and 96 h during the definitive test
- § Exclusion of 0.1 mg/L test concentration from the range-finding test
- § Replicates were not discussed in the range-finding or definitive test
- § In preparation of test solutions for both range finding and definitive tests, the purity value of 95% rather than 99.9% was used in the calculation of required test item weight

**10. SUBMISSION PURPOSE: Registration**

11. **MATERIALS AND METHODS**A. **Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> <ul style="list-style-type: none"> <li>Preferred freshwater species: bluegill sunfish (<i>Lepomis macrochirus</i>) or rainbow trout (<i>Oncorhynchus mykiss</i>)</li> <li>Preferred saltwater species: Atlantic silverside (<i>Menidia menidia</i>) or Sheepshead minnow (<i>Cyprinodon variegatus</i>)</li> </ul>	<ul style="list-style-type: none"> <li>Rainbow Trout (<i>Oncorhynchus mykiss</i>)</li> </ul>
<b><u>Weight</u></b> <ul style="list-style-type: none"> <li>Juvenile fish &lt; 3.0 g</li> </ul>	<ul style="list-style-type: none"> <li>~1-3 g</li> </ul>
<b><u>Length</u></b> <ul style="list-style-type: none"> <li>Longest not &gt; 2x shortest</li> </ul>	<ul style="list-style-type: none"> <li>~4-6 cm</li> </ul>
<b><u>Supplier</u></b>	<ul style="list-style-type: none"> <li>Marian Mill Trout Hatchery, Clwyd, Wales</li> </ul>
<b>All fish from same source and population?</b>	<ul style="list-style-type: none"> <li>Yes</li> </ul>
<b>Fish used in previous tests?</b>	<ul style="list-style-type: none"> <li>No data were available</li> </ul>
<b>If wild fish used, quarantined 7 days before acclimation?</b>	<ul style="list-style-type: none"> <li>Fish were from a hatchery</li> </ul>
<b>Signs of stress or injury?</b>	<ul style="list-style-type: none"> <li>All fish were in good health and free of any apparent malformation</li> </ul>

B. **Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> <ul style="list-style-type: none"> <li>Minimum 12 days (14 days recommended)</li> <li>Minimum 7 days in test dilution water</li> </ul>	<ul style="list-style-type: none"> <li>Fish were acclimated for a minimum of 12 days prior to commencement of the study</li> </ul>
<b><u>Holding Water</u></b> <ul style="list-style-type: none"> <li>Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period)</li> </ul>	<ul style="list-style-type: none"> <li>Reconstituted freshwater, prepared at Inveresk, with high-grade salts and reverse osmosis water was used during holding phase of the study</li> </ul>
<b><u>Disease Treatment</u></b> <ul style="list-style-type: none"> <li>No treatments within 48 hrs of test initiation or during test</li> </ul>	<ul style="list-style-type: none"> <li>No data were available</li> </ul>

Guideline Criteria	Reported Information
<b><u>Feeding</u></b> <ul style="list-style-type: none"> <li>No feeding within 48 hrs of test initiation.</li> <li>Feed daily prior to this period.</li> </ul>	<ul style="list-style-type: none"> <li>During holding, fish were fed on a suitable standard fish diet</li> <li>Fish were not fed for a period of 24 hours prior to commencement of the study or during the tests</li> </ul>
<b><u>Pretest Mortality</u></b> <ul style="list-style-type: none"> <li>&lt; 5% during acclimation; reject entire batch if &gt; 10%.</li> </ul>	<ul style="list-style-type: none"> <li>No data were available</li> </ul>
<b><u>Water Temperature</u></b> <ul style="list-style-type: none"> <li>Temperature changes should not exceed 3°C per day</li> <li>Hold fish minimum 7 days at test temperature prior to testing</li> </ul>	<ul style="list-style-type: none"> <li>Ranged from 15.9-16.5°C for all treatment and control groups</li> <li>No mention of holding fish at test temperature, but were acclimated to laboratory conditions at least 12 days before the study began</li> </ul>
<b><u>Background</u></b> <ul style="list-style-type: none"> <li>During final 48 hrs, colors and light intensities similar to testing area</li> </ul>	<ul style="list-style-type: none"> <li>No data were available</li> </ul>

### C. Test System

Guideline Criteria	Reported Information
<b><u>Dilution Water</u></b> <ul style="list-style-type: none"> <li>Reconstituted water or water from natural source preferred. If dechlorinated tap water, daily chlorine analysis performed.</li> <li>Chemical analysis performed and maximum concentrations not exceeded (see guideline)</li> </ul>	<ul style="list-style-type: none"> <li>Reconstituted freshwater, prepared at Inveresk, with high-grade salts and reverse osmosis water was used during testing phase of the study</li> <li>Appendix 2 of the study displays the results of the analysis of the reconstituted water</li> </ul>

Guideline Criteria	Reported Information
<p><b><u>Solutions</u></b></p> <ul style="list-style-type: none"> <li>Distilled water used to make stock solutions of test substances. If stock volume &gt; 10% of test solution volume, dilution water used.</li> </ul>	<p><i>Range-finding:</i></p> <ul style="list-style-type: none"> <li>Test solutions were prepared by the addition of amounts of CPC (21.05, 210.52, and 2105.11 mg for the 1.05, 10.5, and 105 mg/L solutions respectively) to tanks contains 20 liters of test water.</li> <li>Tanks were ultrasonicated for ~1 min to aid in dissolution of the test item</li> </ul> <p><i>Definitive test:</i></p> <ul style="list-style-type: none"> <li>Test solutions were prepared by appropriate dilution of individual stock solutions, prepared for each concentration</li> <li>Individual stock solutions were prepared by serial dilution (1:1) for a stock solution of CPC (prepared at 84 mg/L nominal)</li> <li>The 84 mg/L stock solution was prepared by adding CPC (84.22 mg) to a flask containing 1 L of test water and ultrasonicated it for <i>ca</i> 2-3 minutes to aid dissolution</li> <li>A 200 mL aliquot of each individually prepared stock solution was added to tanks containing test water (19.8 L) and the tank contents ultrasonicated for <i>ca</i> 1 min to ensure homogeneity</li> <li>Untreated water only was used in the control tank</li> </ul>
<p><b><u>Water Temperature</u></b></p> <ul style="list-style-type: none"> <li>10 or 12 ± 2°C for cold water species (see guideline)</li> <li>22 or 23 ± 2°C for warm water species (see guideline)</li> <li>Vary no more than 1°C in any 24-hr period</li> <li>Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate.</li> </ul>	<ul style="list-style-type: none"> <li>Recorded at 0, 24, 48, 72, and 96 hours in all tanks during the definitive test</li> <li>Range of 15.9 °C-16.5 °C</li> <li>Measured with a YSI 550 A (Yellow Springs Instrument) dissolved oxygen meter with temperature probe</li> </ul>
<p><b><u>pH</u></b></p> <ul style="list-style-type: none"> <li>&gt; 6.0 and &lt; 8.0 for freshwater testing</li> <li>&gt; 7.5 and &lt; 8.5 for marine testing</li> <li>Measured in each replicate at beginning of test and every 24 hrs</li> </ul>	<ul style="list-style-type: none"> <li>Recorded at 0, 24, 48, 72, and 96 hours in all tanks during the definitive test</li> <li>Range of 6.79-7.22</li> <li>Measured with a Sentron Argus X pH meter</li> </ul>

Guideline Criteria	Reported Information
<b><u>Dissolved Oxygen</u></b> <ul style="list-style-type: none"> <li>• Static: &gt; 60% saturation at all times</li> <li>• Flow-through: &gt; 75% saturation at all times</li> <li>• Measured in each replicate at beginning of test and every 24 hrs</li> </ul>	<ul style="list-style-type: none"> <li>• Recorded at 0, 24, 48, 72, and 96 hours in all tanks during the definitive test</li> <li>• Range of 77.5% to 87.7% air saturation value</li> <li>• Measured with a YSI 550 A (Yellow Springs Instrument) dissolved oxygen meter with temperature probe</li> </ul>
<b><u>Total Hardness</u></b> <ul style="list-style-type: none"> <li>• 40 to 180 mg/L as CaCO<sub>3</sub> (freshwater species)</li> <li>• Measured at beginning of each test</li> </ul>	<ul style="list-style-type: none"> <li>• Determined as 78 mg CaCO<sub>3</sub>/L during the definitive test</li> </ul>
<b><u>Salinity</u></b> <ul style="list-style-type: none"> <li>• 20 ± 5ppt (estuarine species)</li> <li>• Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14</li> </ul>	<ul style="list-style-type: none"> <li>• No data were provided</li> </ul>
<b><u>Test Aquaria/Equipment</u></b> <ul style="list-style-type: none"> <li>• Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®)</li> <li>• Test chambers loosely covered</li> </ul>	<ul style="list-style-type: none"> <li>• Researchers used 25 L tanks of molded glass construction and covered with perspex lids to prevent dust contamination and evaporation</li> </ul>
<b><u>Aeration</u></b> <ul style="list-style-type: none"> <li>• Static systems only if &lt; 60% saturation; if aeration used test concentrations measured.</li> <li>• No aeration in flow-through tests</li> </ul>	<ul style="list-style-type: none"> <li>• Tanks were constantly aerated throughout the test period</li> </ul>
<b><u>Type of Dilution System</u></b> <ul style="list-style-type: none"> <li>• Must provide reproducible supply of toxicant</li> </ul>	<ul style="list-style-type: none"> <li>• Serial dilution</li> </ul>
<b><u>Flow Rate</u></b> <ul style="list-style-type: none"> <li>• Consistent flow rate of 6-10 vol/24 hours</li> <li>• Measured at beginning and end of each test</li> <li>• No more than a factor of 10 variation between replicates</li> </ul>	<ul style="list-style-type: none"> <li>• Static delivery system</li> </ul>
<b><u>Biomass Loading Rate</u></b> <ul style="list-style-type: none"> <li>• Static/Static-renewal: ≤ 0.8 g FWF/L</li> <li>• Flow-through: ≤ 0.5 g FWF/L</li> </ul>	<ul style="list-style-type: none"> <li>• No data were available</li> </ul>
<b><u>Photoperiod</u></b> <ul style="list-style-type: none"> <li>• Range from 12D/12N to 16D/8N, with 15 min transition period</li> <li>• Intensity 30 to 100 lm at water surface</li> </ul>	<ul style="list-style-type: none"> <li>• A light cycle of 16 hours light and 8 hours darks was in operation throughout the test using artificial daylight fluorescent tubes</li> </ul>

Guideline Criteria	Reported Information
<b>Solvents</b> <ul style="list-style-type: none"><li>▪ Not to exceed 0.5 ml/L for static or static-renewal tests or 0.1 ml/L for flow-through tests</li><li>▪ Preferred solvents dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol</li></ul>	<ul style="list-style-type: none"><li>▪ No data were available</li></ul>

**D. Test Design**

Guideline Criteria	Reported Information
<b>Range-Finding Test</b> <ul style="list-style-type: none"><li>• If <math>LC_{50} &gt; 100</math> mg/L with 30 fish, then no definitive test required</li></ul>	<ul style="list-style-type: none"><li>• Conducted at nominal CPC concentrations of 0, 1.05, 10.5 and 105 mg/L (0.1 mg/L test concentration was omitted due to results of the initial range finding test)</li><li>• Test solutions were prepared by the addition of weighed amount of CPC to tanks containing 20 liters of test water</li><li>• Tanks were ultrasonicated for <i>ca</i> 1 min to aid dissolution of the test item</li><li>• Control tank had untreated water only</li><li>• 3 fish were added to the 0, 1.05, and 10.5 tanks</li><li>• Within seconds of adding fish to the 105 mg/L tank, fish exhibited a severe reaction to the test item, such as loss of equilibrium and rapid respiration</li><li>• Only 2 fish were added to the 105 mg/L tank and removed after about 1 minute to avoid additional stress</li><li>• 100% mortality in the 105 mg/L test concentration at 0 h</li><li>• At 2 h, all fish were dead in the 10.5 mg/L group</li><li>• At 5 h, all fish were dead in the 1.05 mg/L group</li><li>• No control mortality was observed during the test</li></ul>

Guideline Criteria	Reported Information
<b>Test Concentrations</b> <ul style="list-style-type: none"> <li>Minimum of control and 5 concentrations in geometric series</li> <li>Concentrations 50 to 120% greater than next lowest concentration</li> <li>No more than 25% variation between test concentrations within same treatment</li> <li>Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities (&gt; and &lt; 50%) after 96 hrs</li> <li>Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations</li> </ul>	<ul style="list-style-type: none"> <li>Nominal concentrations of CPC of 0, 0.05, 0.11, 0.21, 0.42 or 0.84 mg/L.</li> <li>Used to produce LC<sub>50</sub> and NOEC</li> </ul>
<b>Concentration Analysis</b> <ul style="list-style-type: none"> <li>Performed at test initiation and every 48 hrs</li> <li>Static: each replicate, minimally at test initiation (before organisms added), at 48 hrs and at end of test</li> <li>Static-renewal: each replicate, at test initiation and end, and just before and after each renewal</li> <li>Flow-through: each replicate at 0, 48, and 96 hrs, and every 96 hrs thereafter</li> </ul>	<ul style="list-style-type: none"> <li>Performed at 0 and 96 hours during the definitive test</li> </ul>
<b>Controls</b> <ul style="list-style-type: none"> <li>Consist of same dilution water, conditions, procedures and test population</li> <li>Negative and/or solvent</li> <li>Maximum allowable mortality 10% (or 1 mortality if 7 to 10 fish used) for 96 hr period; 10% additional past 96 hrs.</li> </ul>	<ul style="list-style-type: none"> <li>Consist of untreated water only</li> </ul>
<b>Replicates</b> <ul style="list-style-type: none"> <li>Two per test concentration</li> <li>Equal volume test solution and number test fish</li> </ul>	<ul style="list-style-type: none"> <li>Replicates were not discussed</li> </ul>

Guideline Criteria	Reported Information
<b>Test Organisms</b>	<i>Range Finding:</i>
<ul style="list-style-type: none"> <li>Minimum 7/replicate (10 preferred)</li> <li>Equal number per test chamber</li> <li>Not fed during treatment period</li> <li>Randomly or impartially assigned to test vessels within 30 min of addition of test substance</li> <li>Biological observations made at 6 hrs and every 24 hours</li> </ul>	\$ 3 fish per tank at each concentration, except 105 mg/L concentration \$ Not fed during the treatment period <i>Definitive Test:</i> \$ 7 fish per tank at each concentration

**12. REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	<ul style="list-style-type: none"> <li>Yes, pages 3,4, and 26 of the report</li> </ul>
Name of test facilities, test dates and personnel reported?	<ul style="list-style-type: none"> <li>Yes, pages 5 and 8</li> </ul>
Identification of test substance (including physicochemical characteristics) and purity provided?	<ul style="list-style-type: none"> <li>Yes, page 9</li> </ul>
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	<ul style="list-style-type: none"> <li>Yes, page 11 and 23</li> <li>Yes, limits reported on page 18</li> </ul>
LC <sub>50</sub> concentration-response curves, LC <sub>50</sub> values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	<ul style="list-style-type: none"> <li>Yes, page 14</li> </ul>
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	<ul style="list-style-type: none"> <li>No</li> </ul>
Any protocol deviations which may have influenced final results of test reported?	<ul style="list-style-type: none"> <li>No</li> </ul>
Raw data included?	<ul style="list-style-type: none"> <li>Yes, pages 17-22</li> </ul>
Signs of abnormal behavior by test fish (if any) described?	<ul style="list-style-type: none"> <li>Yes, page 10 and 13 describe fish in an inverted position and respiring intermittently</li> </ul>
Statistical methods reported?	<ul style="list-style-type: none"> <li>Yes, page 14</li> </ul>

**Dose Response***Range Finding Test*

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish		
			0 hour	2 hour	5 hour
Control	Control	3	0	0	0
1.05	N/A	3	0	0	3
10.5	N/A	3	0	3	3
<sup>1</sup> 105	N/A	2	2	2	2

Note: Data was taken for 0, 2, and 5 hour intervals, not 24, 48, 72, and 96 hours

N/A –data were not available

+ The initial range finding test indicated no mortality at 0.1 mg/L

<sup>1</sup> Only 2 fish were added to this tank because they were observed to have an immediate reaction to the test item. The 2 fish were removed from the tank after ~ 1 min as they were exhibiting loss of equilibrium and difficulty in respiration. This test concentration was assigned a default mortality of 100%.

*Definitive Test*

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish						
			1 h	3 h	6 h	24 h	48 h	72 h	96 h
Control	Control	7	0	0	0	0	0	0	0
0.05	ND	7	0	0	0	0	0	0	0
0.11	ND	7	0	0	0	0	0	0	0
0.21	ND	7	0	0	0	0	6	6	7
0.42	ND	7	0	0	0	7	7	7	7
0.84	NQ (at 96 h)	7	0	0	7	7	7	7	7

ND- Not detected (typical limit of detection =0.27 mg/L)

NQ- Not quantifiable (limit of quantification=0.84 mg/L)

**Statistical Results:** The 48 and 72 hours LC50 values were estimated using the Spearman-Kärber method (Hamilton *et al*, 1977). When no fractional mortality was present, such as at 6, 24, and 96 hours, the estimate of the LC 50 was taken as the arithmetic mean of the 0 and 100% mortality concentrations (Rand and Petrocelli, 1985). The NOEC for observed effects was based on observation of the behavior of the fish and reported on the basis of nominal concentrations of CPC.

**Results Synopsis:**

Duration	LC <sub>50</sub> (mg a.i./L)	95% Upper CI	95% Lower CI
6-hr	0.63	0.84	0.42
24-hr	0.32	0.42	0.21
48-hr	0.17	0.20	0.11
72-hr	0.17	0.20	0.11
96-hr	0.16	0.21	0.11

NOEC through 96 hours = 0.11 mg/L

**Other Effects Observed:** At ~48 hours, one of the surviving fish at 0.21 mg/L was observed in an inverted position on the tank base and respiring only intermittently. The fish was removed to avoid additional distress. After 72 hours of exposure, the surviving fish in the 0.21 mg/L group appeared more lethargic than the control fish. By ~78 hours, the one surviving fish in the 0.21 mg/L group was observed at the tank base in an inverted position. The fish was unable to regain equilibrium and thus removed to prevent further distress. No adverse effects were noted in tanks 0, 0.05, and 0.11 mg/L after 96 hours of exposure.

**13. VERIFICATION OF STATISTICAL RESULTS**

	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
.84	7	7	100	.78125
.42	7	7	100	.78125
.21	7	7	100	.78125
.11	7	0	0	.78125
.05	7	0	0	.78125

THE BINOMIAL TEST SHOWS THAT .11 AND .21 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC<sub>50</sub> FOR THIS SET OF DATA IS .1519868

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\* Results were verified in TOXANAL

**Statistical Method:** Binomial Test

**Results Verification Synopsis:** 96-hr LC<sub>50</sub>: 0.15 mg/L      95% C.I: 0.11-0.21 mg/L  
NOEC: Not available, 0.11 mg/L by observation.

**14. REVIEWER'S COMMENTS:** The study seemed adequate, but did have some limitations. The age of the fish were not discussed at all and the study also lacked evidence of replications, as required by the guidelines. These limitations, though, probably did not influence the outcome of the study, due to the fact the size and weight of the fish were of juvenile size. The quality assurance and GLP papers were given and appropriate acclimation data was provided. The statistical results were also very similar to those given by the TOXANAL program.

**References**

Hamilton, M.A, Russo, R.C., Thurston, R.V. (1977) Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology* **11** 714-719.

Rand, G.M., Petrocelli, S.R. (1985) Statistical analysis..*Fundamentals of Aquatic Toxicology* **5** 110-123.